

ATTACHMENT A

CLAIMS:

1. **(original)** An assay for determining rapamycin or rapamycin analog concentrations in a sample comprising:
 - (i) contacting the sample with PKBP12 protein, or with a rapamycin binding fragment of said PKBP12 protein that maintains the rapamycin binding properties, for a time period and under conditions allowing formation of rapamycin/FKBP12 complex;
 - (ii) contacting the rapamycin/FKBP12 complex with a complex-binding domain of mTOR for a time period and under conditions enabling binding of the complex to said complex-binding domain;
 - (iii) detecting the amounts of said complex-binding domain that is bound to the rapamycin/FKBP12 complex ;
 - (iv) comparing the amounts detected in (iii) to a calibration curve, thereby determining the rapamycin concentrations in the sample.
2. **(original)** The assay of claim 1, wherein said rapamycin being native rapamycin or synthetically produced rapamycin, or any analog of the two.
3. **(original)** The assay of claim 1, wherein said sample is a liquid, a solid or a semi solid sample.
4. **(original)** The assay of claim 3, wherein said liquid sample is a body fluid selected from plasma, blood, serum, urine, sperm, or cerebral spinal fluid.
5. **(original)** The Assay of claim 3, wherein said solid sample is a tissue.
6. **(original)** The assay of claim 3, wherein said sample is semi-solid sample selected from tissues or feces.
7. **(original)** The assay according to claim 4, wherein said liquid sample is mammalian blood.

8. **(original)** The assay according to claim 1, wherein said FKBP12 protein is full FKBP12 protein being a 12kDa protein or a fragment of FKBP12 protein that maintains the rapamycin binding properties of the full protein.
9. **(original)** The assay according to claim 1, wherein the FKBP12 protein or the fragment thereof is immobilized on a solid substrate.
10. **(original)** The assay according to claim 9, wherein said solid support is a 96-well microtiter plate.
11. **(original)** The assay according to claim 10, wherein said microtiter plate is blocked by non specific protein.
12. **(original)** The assay according to claim 1, wherein said detection is achieved by an ELISA reader.
13. **(original)** The assay of claim 1, wherein said complex binding domain of mTOR is a FRB fragment.
14. **(original)** The assay according to claim 13, wherein said FRB fragment is directly bound to a detectable label.
15. **(original)** The assay according to claim 13, wherein said FRB fragment is indirectly bound to a detectable label.
16. **(currently amended)** The assay according to claim 14 ~~or 15~~, wherein said label is capable of generating a signal detectable by a technique selected from coloremtry, spectrophotometry, fluorospectrophotometry, gaseometry or radiospectrometry.
17. **(original)** The assay according to claim 16, wherein said detectable label is an enzyme capable of producing, in the presence of a suitable substrate, a color reaction.
18. **(original)** The assay according to claim 17, wherein said enzyme is alkaline phosphatase or HRP enzyme.
19. **(original)** The assay according to claim 18, wherein said enzyme is used with a color-forming reagent or reagents selected from p-nitrophenyl phosphate, hydrogen peroxide, o-phenylenediamine and 3,3',5,5'-Tetramethylbenzidine.

20. **(original)** A kit for determining rapamycin concentrations, or rapamycin analog concentrations in a sample, the kit comprising:
- (i) PKBP12 protein or a rapamycin binding portion thereof immobilized on a solid substrate; and
 - (ii) a complex-binding domain of mTOR linked to a label that may be detected or that may generate a signal.
21. **(original)** The kit of claim 20, wherein said complex binding domain of mTOR is provided in separate vessel.
22. **(original)** The kit according to claim 20, wherein said complex binding domain of mTOR is the 93-amino acids FRB domain, linked to a label that can be detected or that can generate a signal.
23. **(original)** The kit according to claim 22, wherein said label is capable of generating a signal detectable by a technique selected from coloremtry, spectrophotometry, fluorospectrophotometry, gaseometry or radiospectrometry.
24. **(original)** The kit according to claim 22, wherein said label is capable of producing a colorimetric reaction.
25. **(original)** The kit according to claim 22, wherein said label is an enzyme capable of producing, in the presence of a suitable substrate, a colorimetric reaction.
26. **(original)** The kit according to claim 25, wherein said enzyme is alkaline phosphatase enzyme or HRP enzyme.
27. **(original)** The kit according to claim 22 further comprising antibodies which are conjugated to an enzyme capable of producing a colorimetric reaction.
28. **(original)** The kit according to claim 27, wherein said antibodies are directed against the FRB fragment.
29. **(original)** The kit according to claim 24, wherein said antibodies are directed against a tag to which FRB or FRB fragment are being conjugated.

30. **(original)** The kit according to claim 22 further comprises the label required to generate a signal detectable by a technique selected from coloremtry, spectrophotometry, fluorospectrophotometry, gaseometry or radiospectrometry.
31. **(original)** The kit according to claim 22 further comprising pre-weighed samples of rapamycin and rapamycin analogs for producing calibration curves.
32. **(new)** The assay according to claim 15, wherein said label is capable of generating a signal detectable by a technique selected from coloremtry, spectrophotometry, fluorospectrophotometry, gaseometry or radiospectrometry.